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Sharmila G
P.G and Research Department of Zoology and Wildlife Biology A.V.C. College (Autonomous), Mannampandal – 609 305, Mayiladuthurai Nagapattinam Distict, Tamil Nadu, India

AV Kavitha
Department of Zoology Govt. College for women (Autonomous), Kumbakonam, Tamil Nadu, India

Correspondence
Sharmila G
P.G and Research Department of Zoology and Wildlife Biology A.V.C. College (Autonomous), Mannampandal – 609 305, Mayiladuthurai Nagapattinam Distict, Tamil Nadu, India

Alterations in biochemical parameters of *Cyprinus carpio* (Linn. 1758) Induced by chronic exposure to organophosphorus pesticide, monocrotophos

Sharmila G and AV Kavitha

Abstract
The impact of chronic toxicity of Monocrotophos on the biochemical parameters of freshwater fish, *Cyprinus carpio* has been studied here. Though plenty of research is available for the short term impact of pesticides on the fishes but the studies on the alterations in biochemical parameters due to chronic intoxication has not been carried much. Hence the present study deals with the alterations in biochemical constituents in selected tissues of fishes due to long term exposure to the commonly used agricultural pesticide, monocrotophos. The common edible fish *Cyprinus carpio* were procured and their sublethal, median lethal and lethal concentrations for 120 hrs were assessed as 100 ppm, 155.36 ppm and 250 ppm respectively. For chronic toxicity, 1/10th of LC_{50} for 120 hrs was used as sublethal concentration (15.536 ppm) to which the fishes were exposed for 30 days. The impact of toxicity on the biochemical parameters in muscle, liver and kidney of fishes were assessed at the end of 30 days of exposure to the toxicant and later during the 30 days recovery period. The one ways Anova results showed that treated fishes exposed to the pesticide showed significant differences in the biochemical parameters of both control and treated fishes ($F(2) = 104.39$, $p<0.01$). In the Wilcoxon Signed ranks test the $T = 86.00$ and the two tailed significance p is 0.039. Based on the results of the sign test using SPSS, the biochemical changes induced by the pesticide during exposure period was significantly different than during the recovery period among the different samples ($p = 0.004 < 0.05$)

Keywords: *Cyprinus carpio*, Monocrotophos, sublethal, biochemical

1. Introduction
While the increased application of synthetic fertilizers, insecticides and pesticides in agricultural fields has become the need of the hour to fulfill the hunger of an ever growing population throughout the world, it has also resulted in a high chance of contamination of aquatic ecosystem located in vicinity of such agricultural and industrial areas, through runoff or ground water leaching of a variety of chemicals (Todd and Leuwen, 2002) [16]. Among the different types of pesticides, the organophosphorus pesticides are most preferable because of their low cumulative ability, high insecticidal property, low mammalian toxicity, less persistence and rapid biodegradability in the environment. But again it has resulted in its unquestionable repeated use to get the maximum gain and expected pest damage.

Agricultural pesticides adopt many pathways after leaving their sites of application to be distributed throughout the aquatic environment, not only contaminating it but also affecting the organisms in it by different ways (Ventura *et al.*, 2008) [18]. Most of these insecticides are not very specific in nature (Tarahi Tabrizi, 2001; Honarpajouh, 2003; Bagheri, 2007; Shayeghi *et al.*, 2007; Vryzas *et al.*, 2009; Werimo *et al.*, 2009; Arjmandi *et al.*, 2010) [15, 3, 2, 14, 19, 20, 1] and are found to be highly toxic to non- target organisms that inhabit natural environments close to agricultural fields. Of the various organisms in aquatic environment, the fishes are being most affected partly due to biomagnification as it occupies the higher trophic level of any aquatic food chain but also due to high sensitivity possessing the same biochemical pathway to do with the endogenous and exogenous agent as do the mammalian species (Lackner, 1998: Uner *et al.*, 2006 and Banaee *et al.*, 2008) [9, 17, 4]. Hence insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs and tissues of fish (Banaee *et al.*, 2011) [3]. Since fishes are important sources of proteins and lipids for human forming an integral part of food (Oruce and Usta, 2007) [12],

health of fishes becomes more of concern to human beings. Much works have been done on the short term effect of organophosphorus pesticides on the biochemical, physiological and haematological parameters of fishes. However questions such as that does a prolonged exposure to the sub lethal concentration of pesticides has any effect on the fishes or the steps of checking water contamination by these pesticides will be of any help to the already exposed fishes to retrieve back to its normal status remain unanswered. Keeping in view of such scarce studies, in the present study an effort has been taken to examine the chronic effect of sublethal concentration of Monocrotophos for 30 days on some biochemical parameters of selected tissues of *Cyprinus carpio*.

C. carpio is an economically important fresh water fish and commonly cultured in many parts of the Cauvery deltaic region in village ponds nearby agricultural fields. It is a highly palatable and preferred for culture due to its high growth rate and prolific breeding in confined water. This species has been selected as it is a very important model organism for toxicity tests because of its availability throughout the year and easy acclimatization to laboratory condition. Similarly, the observation on different biochemical parameters in selected tissues of the fishes may provide a very good diagnostic parameter to assess the effect of environmental stress on an animal and the rate of recovery as well.

2. Materials and Methods

2.1 Collection and Acclimatization of the Specimen

Live and healthy *C. carpio* (n = 150) were collected from a commercial fish farm. The fishes were stocked and maintained in aquaria under a natural photo-regimen (14/10 h, light/dark) and at a constant water temperature of 23 ± 10 ^{o}C and a pH of 7-8 to acclimatize for a period of 20 days prior to the experiment. The mean length of the fish was 6.78 cm (range 5.0 to 8.5) and weight was 5.73 gm (range 3.8 to 7.3). During acclimatization, the water was changed daily and the fishes were fed with pellet feed twice a day. Care was taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started.

2.2 Selection of toxicant

Monocrotophos (36% SL), a commonly used organophosphorous pesticide in agricultural fields was used as the toxicant for the present study. This pesticide was collected from a retailer shop in Kuttalam town. It is a systemic insecticides having contact action and widely used against a variety of insect pests from soft bodied insect to leaf eating beetles. It is also effective against borers and bollworms. It has got acaricidal property also. At recommended dosages it is non phytotoxic.

2.3 Physico-Chemical Parameters of the Test medium

Dechlorinated tap water was used as the test medium in the present study. The physico-chemical characteristics of the test medium analyzed following the methods mentioned in APHA (1998) were as follows: Temperature - 23 ± 5 ^{0}C, Dissolved Oxygen (DO) - 6.5 ± 0.15 mg/l, Salinity - 2.7 ± 1 ppt and pH - 7.0 ± 1.

2.4 Toxicity tests

Stock solution of Monocrotophos was prepared from which different concentrations were derived. Ten acclimatized fishes were introduced in each concentration and based on the percentage of mortality the LC_{50} value of Monocrotophos for 120 hrs was assessed using Probit analysis (Finney, 1971) [7]. For biochemical studies, five plastic troughs (two control and three replicates of experiment) having a capacity of 40 L were taken and previously acclimatized 5 fishes were transferred to each trough. Of the five troughs, two were kept as control, without any toxicant and three were contaminated with 1/10th of 120hrs LC_{50} concentration of monocrotophos. The troughs were kept under continuous aeration and food. The experiment was continued for 30 days and dead fish if any were immediately removed.

2.5 Biochemical analysis

At the end of 30 days of exposure period, the fishes from one control and experimental troughs were sacrificed and tissues such as muscle, kidney and liver were extracted for biochemical analysis. By following standard procedures, the total soluble carbohydrates, protein (Lowry *et al.* 1951) [10] and lipid content (Bligh and Dyer 1959) [5] of the muscle, kidney and liver of the normal as well as the Monocrotophos treated fishes were estimated. The results were expressed as mg/g of sample. The other experimental troughs were left untreated for the next 30 days (Recovery period).

2.6 Statistical study

Biochemical parameters were represented as mean ± standard error of mean and the differences in the biochemical parameters among the fish group exposed to sublethal concentrations of monocrotophos were subjected to one-way ANOVA (analysis of variance). Post hoc test were carried out using Duncan multiple comparison test procedure. Significance was tested at $p<0.05$.

When the normality of data did not present, the non-parametric test Wilcoxon – signed ranks test and sign test was used to compare the exposure and recovery data.

3. Results

The mortality of *Cyprinus carpio* exposed to different concentrations of organophosphorus pesticide, Monocrotophos for different hours are shown in Table - 1. Based on the acute toxicity tests, the sublethal (LC_0), median lethal (LC_{50}) and lethal concentration (LC_{100}) of Monocrotophos for 120 hrs in *Cyprinus carpio* were assessed as 100 ppm, 155.36 ppm and 250 ppm respectively. For chronic exposure, fishes were exposed to the sublethal concentration 15.536 ppm ($1/10^{th}$ of 120 hrs LC_{50}) of monocrotophos.

The Fig. 1-3 indicates that the fishes exposed to the sublethal concentration of monocrotophos for 30 days showed considerable variations in biochemical parameters over control. In the treated groups, a decrease in the total soluble carbohydrates was found when compared to the control group. Among the three tissue, the decrease in carbohydrate level was found to be more significant in liver (4.46 ± 0.13 mg/g) than that of muscle (15.36 ± 0.23 mg/g) and kidney (7.23 ± 0.23 mg/g). However during the recovery period, an increase in the total carbohydrate level was found in kidney and liver when compared to the exposure period. The protein content in all the selected tissues viz. muscle, kidney and liver decreased significantly when compared to the control. But during the recovery period, the protein content in kidney (9.03 ± 0.36 mg/g)

showed an increase when compared to the other two tissues (Fig. 2). Similarly the total lipid content also showed a decreasing trend in all the tissues during the exposure period, particularly in liver (0.03 ± 0.00 mg/g). But during the recovery period it showed a significant increase in all the tissues when compared to the exposure period (Fig. 3).

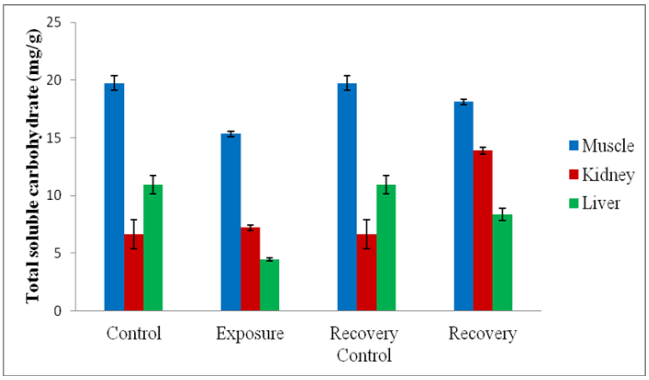

Fig 1: Effect of monocrotophos on the total soluble carbohydrate content in selected tissues of *Cyprinus carpio* during the exposure and recovery period (n = 5) (Mean ± S.E)

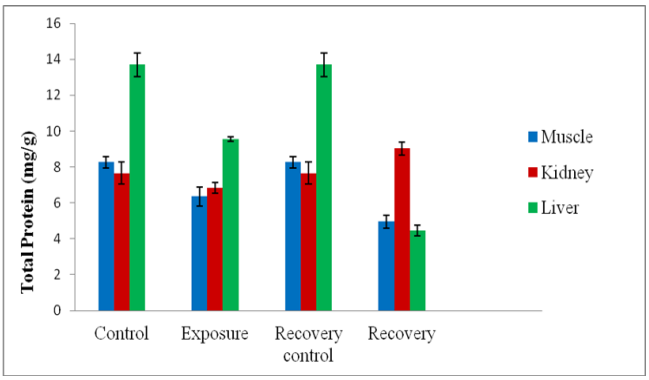

Fig 2: Effect of monocrotophos on the total protein content in selected tissues of *Cyprinus carpio* during the exposure and recovery period (n = 5) (Mean ± S.E)

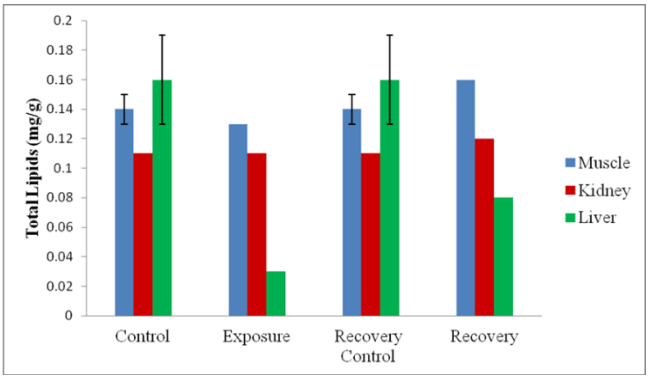

Fig 3: Effect of monocrotophos on the total lipid content in selected tissues of *Cyprinus carpio* during the exposure and recovery period (n = 5) (Mean ± S.E)

Table 1: Results of One way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2605.642	2	1302.821	104.935	.000
Within Groups	1303.632	105	12.416		
Total	3909.274	107			

$p<0.01$

The one way - ANOVA results showed that there was a significant difference between the variations in biochemical parameters of control fishes and that of the fishes exposed to the sublethal concentration of monocrotophos ($F(2) = 104.39$, $p<0.01$) (Table - 1).

Table 2: Wilcoxon signed rank test for biochemical parameters in *Cyprinus carpio* during the exposure and recovery period

	N	Mean rank	Sum of Ranks
Negative ranks	5[a]	17.20	86.00
Positive ranks	20[b]	11.95	239.00
Ties	2[c]		
Total	27		

Test Statistics[a]	
	V8 - V7
Z	-2.061[b]
Asymp. Sig. (2-tailed)	.039
a. Wilcoxon Signed Ranks Test	
b. Based on negative ranks.	

In the Wilcoxon Signed ranks test the T = 86.00 and the two tailed significance p is 0.039. Based on the results from SPSS, the biochemical changes was significantly different (T = 86.00, n = 20, $p<0.05$). In addition, the sum of the positive difference ranks ($\Sigma R+ = 239.00$) was larger than the sum of the negative difference ranks ($\Sigma R- = 86.00$), indicating a significant impact of the pesticide (Table - 2).

Table 3: Sign test for variations in biochemical parameters of *Cyprinus carpio* during the exposure and recovery period

Test Statistics[a]	
	V8 - V7
Exact Sig. (2-tailed)	.004[b]
a. Sign Test	
b. Binomial distribution used.	

Based on the results of the sign test using SPSS, the biochemical changes induced by the pesticide during exposure period was significantly different than during the recovery period among the different samples ($p = 0.004 < 0.05$) (Table - 3).

4. Discussion

To understand the effects of pesticides on fishes there is a great need of testing the toxic effects of more commonly used pesticides on the commercially important fishes. In the present investigation the chronic effect of Monocrotophos on the freshwater fish, *Cyprinus carpio* was studied. To assess the sublethal concentration of monocrotophos for *Cyprinus carpio*, acute toxicity tests were conducted by range finding tests and later full scale tests were conducted to determine the biochemical alterations induced by chronic exposure to the pesticide. In *Cyprinus carpio*, the 100 ppm was found to be sublethal and 250 ppm as lethal concentration for 120 hours. The 120 hours LC_{50} value for *Cyprinus carpio* was observed to be 155.36 ppm. Normally, the rate of concentration of the pesticide is directly proportional to the rate of mortality. At lower concentration, the mortality was restricted but under certain specific environmental conditions such as at 120 hours, even the sublethal concentration of pesticide may be toxic to aquatic environment and organisms like fish etc. At higher concentration, the pesticide increases the rate of impact and

the mortality also increases. Hence it is necessary to assess the impact of pesticides when the fishes are exposed for a prolonged period to even sublethal concentration.

The toxicants present in sub-lethal concentrations in water might enter into the body of the fish *Cyprinus carpio* through the gills or the mucus epithelium of the mouth and finally be distributed in different organs of the body which in turn affects various metabolic pathway. The significant alterations in biochemical parameters may be considered to be manifestation of stress induced by monocrotophos exposure. In agreement with our results, Luskova *et al.*, (2002) [11] reported a significant increase in biochemical parameters in common carp *Cyprinus carpio* following the action of diazinon as the manifestation of stress. Yaji *et al.*, (2011) [21] reported similar findings in fresh water fish *Oreochromis niloticus* exposed to cypermethrin. In the present investigation, the muscle, kidney and liver were selected for the biochemical studies since these are the main target organs for the action of many pollutants and involved in detoxification.

The synchronized fall of carbohydrate level in the muscle of fishes exposed to the toxicant may be due to the expenditure of energy for the constant movements aided by muscular action. Carbohydrates represent the principal and immediate energy precursors for the fishes exposed to stress conditions and a decline in carbohydrate content is attributed to the increased energy demand which could not be compensated by food since the animals are not feeding normally during the experimental period. Similarly there was an overall significant decrease (<0.05) in the protein content in all selected tissues in fishes exposed to the toxicants. The quantity of protein is dependent on the rate of protein synthesis or on rate of its degradation. Renu Bala, (2013) [13] reported that the decline in protein content can be related to impaired food intake, increased energy cost of homeostasis, tissue repair and detoxification mechanisms required during stress as many organisms will mobilize proteins as an energy source via the oxidation of amino acids or induce proteolysis to meet increased energy demand under the conditions of stress. The toxicity of pesticide demands a higher energy supply and that there is a depletion of some energy stores like fat bodies from the tissues like muscles which is the site of rapid protein synthesis. Thus the increased toxicity of pesticides results in variable changes in the protein level of muscles on exposure of monocrotophos compared to control. The observed changes in the total lipid content in *Cyprinus carpio* in this study are indications that the pesticides also affects lipid metabolism in the fish. It was reported in the present study that during Monocrotophos exposure period, the lipid content level in the liver showed a marked decline when compared to the muscle and kidney lipid content. as a consequence of high demand of energy during stress condition and when there is minimum of food intake, the stored energy like lipid is readily used up to meet the energy demand. However during the recovery period results reveals that the biochemical constituents was gradually regained. This observation is in close agreement with the previous observation of Shanthi (2001), who reported an elevation of biochemical constituents during recovery period in Endosulfan pretreated *Cyprinus carpio*.

Therefore, the sum of these alterations in biochemical constituents can have a significant effect on the energy metabolism of fishes exposed to the toxicant while it tends to show a recovery trend also by trying to regain the parameters back to its normal level. In conclusion, the present work indicates that prolonged exposure to sublethal concentration of agrochemicals causes considerable changes in intermediary metabolism and is likely to induce tissue damage in *Cyprinus carpio.* The causes for these alterations appear to be the result of high energy demands.

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